

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Dale B. Schenk

Application No.: 09/723,713

Filed: November 27, 2000

For: PREVENTION AND TREATMENT
OF AMYLOIDOGENIC DISEASE

Customer No.: 20350

Confirmation No. 9870

Examiner: Anne Marie Sabrina Wehbe

Technology Center/Art Unit: 1632

Declaration of Dr. J. Steven Jacobsen under
37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, J. Steven Jacobsen, state as follows.

(1) My current position is Associate Director, Neuroscience, Wyeth Research, Princeton, NJ 08543. I understand Wyeth is a licensee of the above-captioned application. A copy of my curriculum vitae is attached.

(2) I am familiar with the publication Bard et al., Proc. Natl. Acad. Sci. USA 100, 2023-2028 (2003), which reports that the 10D5 antibody, despite significantly lowering levels of A β in the brain of a transgenic mouse model of Alzheimer's disease, did not significantly reduce neuritic dystrophy.

(3) I understand that the Examiner of the above-captioned application interprets the above result as evidence that the 10D5 antibody would not be useful in treating Alzheimer's disease.

(4) I and/or others acting under my supervision have performed experiments to test the 10D5 antibody for effects on cognition in a transgenic mouse model of Alzheimer's disease. In brief, the experiments show that the 10D5 antibody does have a statistically significant beneficial effect on cognition notwithstanding the lack of such an effect on neuritic dystrophy reported in Bard et al..

(5) The details of the experiments are as follows.

METHODS & MATERIALS

Animals:

Transgenic (Tg2576) mice were heterozygous for the K670N/M671L amyloid precursor protein transgene. All transgenic genotypes were confirmed by PCR and all animals homozygous for the Retinal Degeneration (Rd) mutation were excluded. The background strain consisted of a C57Bl6 and 129SJL cross.

Transgenic mice exhibited cognitive deficits in contextual memory beginning at 14-16 weeks of age. Cognitive deficits were particularly prominent at 20 weeks of age and were maintained up to 65 weeks of age.

Testing Apparatus

Approximately 20 week old male transgenic mice and wild-type littermate control mice were individually housed for at least 2 weeks prior to any testing and allowed *ad libitum* access to food and water. CFC occurred in six 30 x 24 x 21cm operant chambers (Med Associates, Inc) constructed from aluminum sidewalls and plexiglass ceiling, door and rear wall. Each chamber was equipped with a floor consisting of 36 stainless steel rods through which a foot shock could be administered. In addition, each chamber had 2 stimulus lights, one house light and a solenoid. Lighting, the footshock (US) and the solenoid (CS) were all controlled by a PC running MED-PC software. The chambers were located in a sound isolated room in the presence of red light.

Contextual Fear Conditioning (CFC) Assay

Mice (n=8-12/genotype/treatment) were trained and tested on two consecutive days. The Training Phase consisted of placing the mice in the operant chambers, illuminating both the stimulus and houselights and allowing them to explore for 2 minutes. At the end of the two minutes, the auditory cue (2Hz clicking via the solenoid; CS) was presented for 15 seconds. The footshock (US; 1.5 mAmp) was administered for the final 2 seconds of the CS and co-terminated with the CS presentation. This procedure was repeated and 30 seconds after the second foot shock the mice were removed from the chambers and returned to their home cages.

Twenty hours after training, animals were returned to the chambers in which they had previously been trained. Freezing behavior, in the same environment in which they had received the shock ("Context"), was then recorded by the experimenter using time sampling in 10 seconds bins for 5 minutes (30 sample points). Freezing was defined as the lack of movement except that required for respiration. At the end of the 5 minute Context test mice were returned to their homecages. Freezing in the Novel and Cue condition was collected after all of the mice had been tested in Context (~60 minutes after the initial Context test). The novel environment consisted of modifications of the operant chamber including an opaque plexiglass divider from the rear right corner to the front left, a plexiglass floor as well as decreased illumination (housetlight only). Mice were placed in the Novel context and time sampling was used to collect freezing scores for 3 minutes (18 sample points). At the end of the 3 minutes, the auditory clicker (CS) was presented for 3 minutes during which freezing was again scored (18 sample points). Freezing scores for each animal were converted to percent freezing for each portion of the test. Memory for the context (Contextual memory) for each animal was obtained by subtracting freezing score in the novel condition (a measure of basal activity) from that observed in the context.

Treatment Regime

Wild-type and Tg2576 mice were administered a single dose of phosphate buffered saline (PBS) or treatment antibody by intraperitoneal injection at 24 hours prior to the training phase of the CFC.

RESULTS

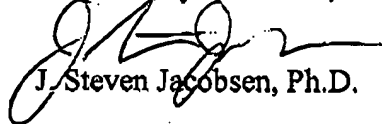
Therapeutic efficacy is expressed both in terms of memory deficit reversal and memory impairment status. "Memory deficit reversal" was determined by comparing the freezing behavior of mAb- vs. PBS control-treated Tg2576 animals. "Memory impairment status" was determined by comparing the freezing behavior of wild-type vs. Tg2576 mAb-treated animals. The results indicated that the mAb 10D5 (at 30 mg/kg) caused a significant improvement in contextual memory of Tg2576 mice relative to a control treatment (p value <0.05), and no significant memory impairment with respect to wild-type mice (p value > 0.05).

Memory Deficit Reversal per Ab dosage (mg/kg) (p value WRT PBS Control)					Impairment Status per Ab dosage (mg/kg) (p value WRT WT mice)				
0.3	1	3	10	30	0.3	1	3	10	30
		0.7045	0.9661	0.0189			0.0009	0.002	0.0752

(6) I interpret these data as showing that an antibody to A β can exert a beneficial effect on cognition and thus be useful in treating Alzheimer's disease.

(7) I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,


J. Steven Jacobsen, Ph.D.

Date 10/19/05

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History:

Wyeth Research 07/1995 - Present
Princeton, NJ, USA

Associate Director, Neurodegeneration Research 08/2001 - Present

Team Leader, Alzheimer's Disease Amyloid Programs 07/1995 - Present

Lederle Laboratories, American Cyanamid 05/1988 - 06/1995
Pearl River, NY, USA

Team Leader, Alzheimer's Disease Program 07/1992 - 06/1995

Senior Scientist II, Alzheimer's Disease Program 01/1991 - 06/1992

Senior Scientist I, Alzheimer's Disease Program 12/1989 - 12/1990

Postdoctoral Scientist, Alzheimer's Disease Program 05/1988 - 11/1989

1995-Pres. Wyeth Neuroscience, Wyeth, Princeton, NJ.

Research Interests: Disease-modifying strategies for the treatment of patients with Alzheimer's Disease.

1988-1995 Central Nervous System Biological Research Department, Medical Research Division, Lederle Laboratories, American Cyanamid Co., Pearl River, NY.

Research Interests: Molecular mechanisms of Alzheimer's Disease: Expression and processing of Amyloid Precursor Proteins.

1988-1989 Postdoctoral Scientist (Molecular Neurobiologist & Biochemist)

1989-1995 Senior Research Scientist II (Molecular Neurobiologist)

1979-1983 Department of Dermatology, New York University School of Medicine, NYU Medical Center, NY, NY.

Research Interests: Identification & purification of human melanoma-associated cell-surface proteins and the development of a vaccine for immunotherapy.

1979-1980 Research Technician I

1980-1981 Research Technician II

1981-1982 Senior Research Technician

Education:

Graduate, Ph.D. in molecular genetics, Dr. M. Zafri Humayun, Dept. of Microbiology and Molecular Genetics, Graduate School of Biomedical Sciences, University of Medicine and Dentistry-New Jersey Medical School, Newark, NJ, 1983 - 1988.

Graduate, M.S. in biochemistry, Dr. John R. Keller, Department of Biology, Seton Hall University, South Orange, NJ, 1977 - 1979.

Undergraduate, B.S. in biology, Department of Biology, Seton Hall University, South Orange, NJ, 1974 - 1977.

Invited Lectures:

- 1989 **"Molecular Mechanisms of Amyloid Peptide Precursor in Alzheimer's Disease."** New Jersey Neuropsychopharmacology Society Annual Symposium, Princeton University, Princeton, NJ.
- 1989 **"Alzheimer's Disease and the Molecular Mechanisms of Amyloid Peptide Precursor Expression."** Graduate Biology Seminar Series, Department of Biology, Seton Hall University, South Orange, NJ.
- 1990 **"Expression of Amyloid Peptide Precursor Expression and deposition of β -amyloid peptide in Alzheimer's Disease."** Department of Biology, William Paterson College, Wayne, NJ.
- 1992 **"Processing of β -amyloid peptide in Alzheimer's Disease."** Department of Biology, William Paterson College, Wayne, NJ.
- 1993 **"Molecular Mechanisms of Alzheimer's Disease: Therapeutic approaches to reduce the formation of β -amyloid peptide."** Graduate Biology Seminar Series, Department of Biology, Seton Hall University, South Orange, NJ.
- 1994 **"Alzheimer's Disease and β -amyloid peptide: Molecular Mechanisms to Block the Formation of β -Amyloid Peptide."** Dept. of Molecular Biology and Molecular Genetics, University of Medicine and Dentistry-New Jersey Medical School, Newark, NJ.
- 1995 **"Alzheimer's Disease and β -amyloid peptide: Molecular Mechanisms to Drug Discovery."** Dept. of Environmental Medicine, NYU School of Medicine, Tuxedo, NY.
- 1995 **"Therapeutic Strategies to Reduce Formation of β -amyloid peptide in**

Alzheimer's Disease. Alzheimer's Disease: Latest Advances in Understanding and Treatment. Sponsored by IBC USA Conferences Inc., Waltham, Massachusetts.

- 1996 **"Therapeutic Strategies to Reduce Formation of β -amyloid peptide in Alzheimer's Disease."** Alzheimer's Disease: Advances in Diagnostics and Drug Development. Sponsored by IBC USA Conferences Inc., Oak Brook, Illinois.
- 1999 **"Advances in Understanding the Molecular Mechanisms of Alzheimer's Disease."** Depts. of Environmental Medicine and Biology, NYU School of Medicine, NY, NY.
- 2000 **"Alzheimer's Disease: Development of Protease Inhibitors as A β -Lowering Agents."** American Home Products Corporation, Senior Managers Symposium, Baltimore, MD.
- 2001 **"Alzheimer's Disease: Current and Emerging Therapeutic Strategies."** *Medicinal Chemistry*, Gordon Research Conferences, Colby-Sawyer College, New London, NH.
- 2001 **"Strategic Approaches for the Treatment of Alzheimer's Disease."** Lecture series in Medicinal Chemistry, Associated Colleges of the Chicago Area (ACCA), Morton Arboretum, Lisle, IL.
- 2004 **"Alzheimer's Disease And Emerging New Disease-Modifying Therapeutic Strategies."** The 2004 Robert S. Rozman Memorial Symposium, Delaware Valley Drug Metabolism Discussion Group, Langhorne, PA.
- 2005 **"Anti-Amyloid Approaches for the Treatment of Alzheimer's Disease."** 7TH International Neurodegeneration In Alzheimer's Disease, Parkinson's Disease and Related Disorders, Strategic Research Institute, Princeton, NJ. (April 2005)
- 2005 **"Alzheimer's Disease: The Next Generation of Symptomatic and Disease Modifying Therapies."** Bio2005, Philadelphia, PA. (June 2005)
- 2005 **"Disease-Modifying Therapeutic Approaches for the Treatment of Alzheimer's Disease."** Therapeutic Strategies Against Neurodegenerative Conditions, Boston, MA. (Oct. 2005)

Professional Membership, Review Committees and Appointments:

- 1991-present** Society for Neuroscience
Chicago, Il
- 1993-present** AD HOC Review Committee Member
Alzheimer's Disease Center Cores (ADCCs)
National Institute on Aging (NIA/NIH), Bethesda, Md.
- 1994-present** External Peer Review Committee Member
UMDNJ and Allied Health Services Foundation
University of Medicine and Dentistry-New Jersey Medical School, New Brunswick, NJ.
- 1995-present** Adjunct Associate Professor (Lecturer in Neurobiology and Neurotoxicology; Graduate Student Thesis Committee Member)
New York University School of Medicine, NYU Medical Center, NY, NY.
- 1997-present** Review Board Committee Member,
Medical and Scientific Advisory Council,
Alzheimer's Association, Inc., Chicago, Il,
- 2003-Present** *Current Alzheimer Research*, Editorial Board (Bentham Press)

Bibliography:**Publications:**

1. Bystryn, J.-C., **Jacobsen, J.S.**, Liu, P. and Heaney-Kieras, J. (1982). Comparison of cell-surface human melanoma-associated antigens identified by rabbit and murine antibodies. *Hybridoma*, **1**, 465-472.
2. Refolo, L.M., Conley, M.P., Sambamurti, K., **Jacobsen, J.S.** and Humayun, M.Z. (1985). Sequence context effects in DNA replication blocks induced by aflatoxin B1. *Proceedings of the National Academy of Sciences, USA*, **82**, 3096-3100.
3. Bystryn, J.-C., **Jacobsen, J.S.**, Harris, M., Roses, D., Speyer, J. and Levin, M. (1986). Preparation and characterization of a polyvalent human melanoma antigen vaccine. *Journal of Biological Response Modification*, **5**, 211-224.
4. **Jacobsen, J.S.** and Humayun, M.Z. (1986). Chloroperbenzoic acid induced DNA damage and peracid activation of aflatoxin B1. *Carcinogenesis*, **3**, 491-493.
5. **Jacobsen, J.S.**, Refolo, L.M., Conley, M.P., Sambamurti, K., and Humayun, M.Z. (1987). DNA replication-blocking properties of adducts formed by aflatoxin B1-2,3-dichloride and aflatoxin B1-2,3-oxide. *Mutation Research*, **179**, 89-101.
6. Sambamurti, K., Callahan, J., Luo, X., Perkins, C.P., **Jacobsen, J.S.** and Humayun, M.Z. (1988). Mechanisms of mutagenesis by a bulky DNA lesion at the guanine-N7 position. *Genetics*, **120**, 863-873.
7. **Jacobsen, J.S.**, Perkins, C.P., Callahan, J.T., Sambamurti, K. and Humayun, M.Z. (1989). Mechanisms of mutagenesis by chloroacetaldehyde. *Genetics*, **121**, 213-222.
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specific RNA related to alternatively spliced amyloid precursor protein mRNAs. *Neurobiology of Aging*, **12**, 575-583.

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Book Chapters:

1. Donnelly, R.J., **Jacobsen, J.S.**, Rasool, C.G., Bartus, R., Blume, A.J. and Vitek, M.P. (1989) Isolation and expression of multiple forms of beta amyloid protein precursor cDNA in Alzheimer's Disease. In: *Alzheimer's Disease and Related Disorders. Progress in Clinical and Biological Research, Volume 317*. K. Iqbal, H.M. Wisniewski and B. Winblad (editors), Alan R. Liss, Inc., New York. pp. 925-937.

Abstracts:

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2. Refolo, L.M., Conley, M.P., Sambamurti, K., **Jacobsen, J.S.**, and Humayun, M.Z. 1985. Sequence context effects in DNA replication blocks induced by aflatoxin B1. *Abstracts of the 13th International Congress of Biochemistry*, Amsterdam, The Netherlands, 25-30 August, 1985.
3. Refolo, L.M., Conley, M.P., Sambamurti, K., **Jacobsen, J.S.**, and Humayun, M.Z. 1985. Sequence context effects in DNA replication blocks induced by aflatoxin B1. *Abstracts of the Mechanism of Mutagenesis: Impact on Carcinogenesis*, Mittelwihr, France, 22-27 September, 1985.
4. **Jacobsen, J.S.**, Refolo, L.M., Sambamurti, K. and Humayun, M.Z. 1986. Replication blocks induced by aflatoxin B1: DNA sequence and conformation specificity and

- effect of manganese. UCLA Symposia on Molecular and Cellular Biology: Mechanisms of DNA Replication and Recombination, *Journal of Cellular Biochemistry, Supplement 10B*, Park City, Utah, 22 February-23 March, 1986.
5. **Jacobsen, J.S.** and Humayun, M.Z. 1986. Chloroacetaldehyde-induced DNA lesions block DNA replication and permit misincorporation at A and C residues. *Abstracts of the annual meeting of the Environmental Mutagen Society*, Washington, D.C., 21-23 April, 1986.
6. **Jacobsen, J.S.** and Humayun, M.Z. 1986. Effect of chloroacetaldehyde-induced lesions on DNA template activity. New Jersey State Commission on Cancer Research: *A Workshop on Cancer Research in New Jersey*, Woodbridge, New Jersey, 22 November, 1986.
7. Sambamurti, K., Callahan, J.T., Perkins, C.P., **Jacobsen, J.S.** and Humayun, M.Z. 1988. Activated aflatoxin induces transversions and transitions with equal efficiencies in phage M13. UCLA Symposia on Molecular and Cellular Biology: Mechanisms and Consequences of DNA Damage Processing. *Journal of Cellular Biochemistry, Supplement 12A*, Taos, New Mexico, 24-31 January, 1988.
8. Donnelly, R.J., **Jacobsen, J.S.**, Rasool, C.G., Bartus, R., Blume, A.J. and Vitek, M.P. 1988. Isolation and expression of multiple forms of beta amyloid protein precursor cDNA in Alzheimer's Disease. *Abstracts of Proceedings of the First International Conference on Alzheimer's Disease and Related Disorders*. Las Vegas, Nevada, September, 1988.
9. Vitek, M.P., **Jacobsen, J.S.**, Donnelly, R.J. and Blume, A.J. 1989. Quantitative and qualitative analysis of multiple APP RNA forms in normal and Alzheimer's Brains. UCLA Symposia on Molecular and Cellular Biology: Molecular Biology of Aging. *Journal of Cellular Biochemistry, Supplement 13C*, p. 166, Santa Fe, New Mexico, 4-10 March, 1989.
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brain is species specific and may encode a novel soluble protein. UCLA Symposia on Molecular and Cellular Biology: Molecular Neurobiology. *Journal of Cellular Biochemistry, Supplement 14F*, p. 59, South Padre Island, Texas, 17-23 April, 1990.

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14. Vitek, M.P., **Jacobsen, J.S.**, Sonnenberg, J., Sahasrabudhe, S., Muenkel, H.A., Spruyt, M.A., and Blume, A.J. 1991. The APPREP System, Protein Processing and BAP Production: Characterization of APP-Reporter Protein. *Society for Neuroscience Abstracts*, vol. 17, p. 1443. New Orleans, Louisiana, 10-15 November, 1991.
15. Sonnenberg, J.L., Sahasrabudhe, S.R., Hulmes, **Jacobsen, J.S.**, Blume, A.J. and Vitek, M.P. 1992. N-terminal cleavage of β -amyloid by Cathepsin G is dependent on pH and ionic conditions. *Society for Neuroscience Abstracts*, vol. 18, p. 1441. Anaheim, California, 25-30 October, 1992.
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